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COMPARISON OF SPECIES SENSITIVITY DISTRIBUTIONS BASED ON POPULATION OR INDIVIDUAL ENDPOINTS

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Abstract-Species Sensitivity Distributions (SSDs) developed from individual and population endpoints were compared based on simulations and a case study. The simulations were performed with five invertebrate species accounting for the diversity of benthic macroinvertebrate communities in large European lowland rivers and for five benthic invertebrates used as laboratory species. Population growth rate EC10 values were, in most of the simulations, higher than the lowest of the EC10 values at individual level. However, for the set of ecologically representative species, HC5 were in majority more protective for population endpoints than for individual endpoints. This was the opposite for the set of laboratory species. Population and individual SSDs were also compared based on existing data on copper for the five laboratory invertebrate species. In this case, the calculated population HC5 value was almost twice the individual value, and we showed much reduced variability between species sensitivities at population level compared to individual level. **We conclude that population-based HC5 would generally be more protective than individual-based HC5. However, the change of level could reveal higher homogeneity at population level than at individual level, supporting the use of population-based HC5 to avoid overprotection. We thus advise the derivation of population-based HC5, as soon as it is possible to derive such value with a relevant panel of species.**

Keywords-Species sensitivity distributions Population modelling Invertebrates Computer modelling Ecological risk assessment

INTRODUCTION

In practice, aquatic ecosystem quality standards are set from compilations of standard single-species toxicity tests results. When enough data are available, species sensitivity distributions (SSDs) can be developed from these compilations. SSDs are cumulative distribution functions estimated from toxicity data obtained for some species' responses and are statistically considered a sample of the responses of all the species composing a community. The fifth percentile level of this distribution (HC5) is the most commonly used value in the practice of using SSDs to set environmental predicted no effect concentrations (PNECs) [1].

To be useful in ecological risk assessment, the biological responses considered in the SSD should be ecologically relevant. In practice, the vast majority of SSDs are built from acute data. A step towards more ecologically relevant SSDs consists in using chronic responses. However, chronic effects on survival, reproduction, or growth can induce very different consequences at population level among species [2]. This is generally ignored, and chronic SSDs are generally derived with data relative to a mixture of endpoints, for instance growth and reproduction, keeping the most sensitive endpoint per species to achieve a conservative estimation [3]. Even when only reproduction is investigated, many sub-endpoints are considered, like sex ratio, development, fecundity or egg masses [4].

Using a same endpoint for all species, which would not depend on test duration, could improve the relevance of the SSD approach. This endpoint could be the population growth rate, which has the additional advantage of proposing an endpoint closer to ecosystem level than individual endpoints and able to integrate many effects (on survival, growth and reproduction) in one single parameter. Population level modeling decreases uncertainty associated with standard toxicity assessment through the prediction of the demographic consequences of the effects of chemicals [5].

In this paper, we present different simulation studies and one case study on copper data to study the application of the SSD methodology to the EC10 for population growth rate and compare it with SSD based on individual endpoints. The simulations were performed first with five invertebrate species, accounting for the diversity of benthic macroinvertebrate communities in large European lowland rivers, then with five different benthic macroinvertebrate laboratory species, for which copper data were available.

MATERIALS AND METHODS

Simulated data

We compared SSD obtained for individuals and those obtained at population level through 1000 simulations of dose response relationships for survival, growth and reproduction of five invertebrate species accounting for the diversity of benthic macroinvertebrate communities in large European lowland rivers [6]. These species were *Chironomus riparius* (Insecta: Diptera), *Branchiura sowerbyi* (Oligochaeta: Tubificidae), *Lumbriculus variegatus* (Oligochaeta: Lumbriculidae), *Valvata piscinalis* (Gastropoda: Valvatidae), and *Sericostoma personatum* (Trichoptera: Sericostomatidae).

The life cycle parameters values in control conditions, which we need for population modelling (juvenile survival (S_j), adult survival (S_a), age at first reproduction (t_j), time between reproduction events (t_a) and fecundity (F)), are available in the literature for four out of the five species considered (Table 1). *Chironomus riparius* is a semelparous species with fecundity around 100 females per female and around two weeks to reach maturity [7]. *Branchiura sowerbyi* is a hermaphroditic freshwater worm with, in the laboratory, low hatching rate (32%), high juvenile survival (>80%) and a lifespan up to 1100 days [8]. Reproduction of *Lumbriculus variegatus* under laboratory conditions is by asexual fragmentation, during which a worm spontaneously divides into two or more body fragments

[9]. We assumed it takes about 10 days for individuals to divide in two parts and be able to reproduce again [10]. *Valvata piscinalis* is a gastropod with high hatchability and survival rates in laboratory conditions [11]. For *Sericostoma personatum*, only growth and hatching data is available [12]. This species was replaced by another trichoptera species, *Cheumatopsyche brevilineata* (Trichoptera: Hydropsychidae) for which we had enough data to cover all life cycle parameters, and which has already been proposed for ecotoxicity tests [13].

The dose effect relationship was modeled as a Hill model:

$$\frac{E}{E_{max}} = \frac{C^n}{EC_{50}^n + C^n} \quad (1)$$

with C the exposure concentration, EC_{50} the concentration leading to 50% of effect, and n the slope value.

We performed 1,000 simulations, for the five species, simulating independently at each simulation one dose effect relationship for each endpoint and each species. The effects we considered were a decrease of juvenile survival, a decrease of fecundity and a decrease of the growth rate (resulting in an increase of age at first reproduction). Effects on growth and on reproduction had each 50% of probability of occurrence (which means that there was 25% probability that both occurred, and the same probability that none occurred). We performed two sets of simulations with slope values of $n=1$ and $n=4$ accounting for most of the values found for the copper case study (See Table 2), to which we compared the outcomes of the simulations. The values for n were fixed in each set to assess the influence of the slope value on our conclusions. Different lognormal distributions were used for the EC_{50} values of all the endpoints. The distributions were the same for growth (age at first reproduction) and reproduction (intervals containing 90% of the values were [0.1; 10]), with higher mean values for juvenile survival (the interval containing 90% of the values was [0.5; 50]) assuming that lethal effects, even for juveniles would generally occur at higher exposure concentrations than

sublethal effects. We excluded effects on adult survival, assuming that such effect would occur at high exposure concentrations, and effects on the interval between two reproduction events for such effects are rarely reported in toxicity tests which usually focus on the total number of offspring throughout time.

For each simulation, we calculated SSD on individuals based on the lowest EC10 value among all the effects obtained for each species and SSD on populations based on the EC10 on the population growth rate of each species. To calculate this EC10, we used a generic population dynamics model [14], with a time step of one day:

(2)

The population intrinsic growth rate r is the logarithm of λ . We calculated the dose response relative to r and could estimate the concentration leading to a 10% reduction compared to control value.

To determine the HC5, *i. e.* the value of the 5th percentile of the lognormal distribution that is fitted to the species effect concentrations [14], the EC10 values were ordered from the smallest to the largest value. The ordered values were then given a rank using the equation provided by Clements and Newman [15]:

(3)

where i is the i^{th} ranked observation and N the total number of observations. A lognormal model was assumed and the probit transformation of each rank was done. Hence, a linear model could be fitted between the logarithm of the effect concentrations and the probit of the ranks. Simulation and statistical analysis were performed under the R environment [16].

Application to experimental data

We used the data published by Roman et al. [17]. The authors have previously used these data to derive SSD and calculate HC5 values. These data are composed of reproduction and biomass production for *Lumbriculus variegatus*, growth and reproduction for *Tubifex tubifex*,

growth and emergence for *Chironomus riparius*, growth for *Gammarus pulex* and *Hyaella azteca*, and survival for all species. Authors provide EC10 and EC50 values, which we used to reconstruct the dose response with the Hill model for all endpoints measured for each species. The slope values (n) of the model regression are shown in Table 2.

The biological and ecological characteristics of the five species are available in Table 3. We already presented *Lumbriculus variegatus* and *Chironomus riparius* species. For *Tubifex tubifex*, we used data from Bettinetti and Provini [18]. They used sexually mature worms at their first reproductive event (approximately six weeks) and monitored reproduction after 28 days. McCahon and Pascoe [19] showed that *Gammarus Pulex* needed 130 days to reach sexual maturity, with 70% of organisms surviving until maturity. Cold and Forbes [20] found a mean number of offspring of 13 per female over a 13 days period with 80% of the females reproducing. For *Hyaella azteca*, Péry et al. [21] found a mean reproduction around 14 over a 28 days period, young survival around 0.9 and maturity was reached after 30 days. In a first step, we performed simulations with these five species in the same way as previously to assess the differences in HC5 derivations depending on the choice of species. Then we calculated the HC5 for these five species exposed to copper.

RESULTS

For the simulations with the ecologically representative species in Table 1, the lowest EC10 value at individual level was lower than the EC10 value at population level in 73.8% of the simulations (5,000 values) with $n=1$, and in 62.9% of the simulations with $n=4$ (5,000 values). The comparison between individual and population endpoints was modified for HC5. Indeed, HC5 calculated for the population growth rates EC10 was lower than the HC5 based on the lowest EC10 values in 56.9% of the simulations (1,000 values) with $n=1$, and in 60.9% of the simulations with $n=4$ (1,000 values). This modification when going from EC10 to HC5

values is due to the slope of the SSD regressions. Indeed, in respectively for $n=1$ and $n=4$, 91.5% and 91.6% of the simulations, the slope of the SSD regression was lower for population endpoints compared to individual ones, indicating more variability between species endpoints at population level than at individual level. The ratios between population and individual HC5 values were in the interval [0.2; 5] in 84% of the cases for $n=1$, and in 82% of the cases for $n=4$.

For the simulations with the species in Table 3, the lowest EC10 value at individual level was lower than the EC10 value at population level in 89.7 % of the simulations (5,000 values) with $n=1$, and in 74% of the simulations with $n=4$ (5,000 values). The HC5 calculated for the population growth rates EC10 was lower than the HC5 based on the lowest EC10 values in 44.3% of the simulations (1,000 values) with $n=1$, and in 37.8% of the simulations with $n=4$ (1,000 values). In 85.2% and 75.7% of the simulations respectively for $n=1$ and $n=4$, the slope of the SSD regression was lower for population endpoints compared to individual ones. The ratios between population and individual HC5 values were in the interval [0.2; 5] in 81% of the cases for $n=1$, and in 100% of the cases for $n=4$.

For the calculations based on the copper toxicity data, EC10 values at population level are presented in Table 2. Population and lowest individual EC10 values are close, with at most two fold of differences. There is also less variability between species among EC10 population values than among EC10 individual values.

Figure 1 and Figure 2 present the population growth rates of the five species as a function of exposure concentration together with the SSD for individuals and populations. The HC5 for individuals was estimated at 27.8 mg/kg, and the HC5 for populations at 44.4 mg/kg. The slope of the SSD regression for individual SSD was 2.1. It was 3.5 at population level.

DISCUSSION

In our simulations, populations EC10 values were most of the time higher than the lowest EC10 at individual level, but the slope of the regression to derive the SSD was generally lower at population than at individual level. In general, HC5 values derived with the ecologically relevant species were lower at population level compared to individual level (this was the case in around 60% of the simulations). This was the opposite for the species used for the copper study (lower population-based HC5 than individual-based HC5 in around 40% of the simulations). One explanation for this slight difference would be that the current species used in routine laboratory tests have low variability between their life history strategies compared to species representing actual communities. Indeed, selected laboratory species have short life cycle (rapid growth) and high fecundity resulting in a high population growth rate. **In general (more than 80% of the simulations), individual and population HC5 values did not differ by more than a factor of 5. This factor of 5 is the highest safety factor recommended to derive a PNEC from a SSD [1].**

In the copper case study, population HC5 value was calculated as 1.6 fold the individual value. This was a direct consequence of a high slope of the SSD description at population level, indicating that there was not much difference between species sensitivities at population level, as the slope accounts for the extent of variability among species. The ratio between population and individual slopes belongs to the part of the distribution below 5% for the simulations both with the species of Table 1 and of Table 3 with either $n=1$ or $n=4$, indicating much lower variability among population endpoints between the species exposed to copper than expected based on their life-cycle traits values only. We propose the following explanation. In our simulations, we did not introduce any correlation between sensitivity of life cycle parameters and the sensitivity of the population dynamics to these parameters. Organisms' sensitivity to stress is a function of their biology, and can be predicted from species traits [22]. It is likely that in case of pollutants present widely in the environment,

organisms are adapted from long to this pollutant. Hence, phenotypes more protective for the life history traits with the greater impact on their fitness (and hence on population dynamics at the population level) were selected in the past. For instance, the sensitivity of *C. riparius* life cycle parameters to copper is exactly in the opposite order as the sensitivity of population dynamics to these parameters [23-24]. Similarly, the endpoint impacted at the lowest zinc concentration for the species *Valvata piscinalis* is one of the endpoint for which population dynamics is the less sensitive [25]. **More generally, it has been shown that changes in traits having a high sensitivity to chemical toxicants have a relatively low impact on population growth rate, compared to other life-cycle traits [26].** Our conclusions can obviously not be easily generated because they rely on one chemical only, copper, which is a metal with much specificity relative to acclimatization, bio-availability, and essentiality.

The question of using acute or chronic data to derive ecologically-relevant SSD is an important issue. It has been shown for many substances, including metals, pesticides, organic and inorganic compounds, that HC5 calculated based on laboratory-generated single-species chronic studies are protective of model ecosystem [27-29]. Raimondo et al. [30] evaluated the protectiveness of SSD first and fifth percentile hazard concentrations (HC1, HC5) relative to the application of safety factors using 68 SSDs generated from 1,482 acute (lethal concentration of 50%, or LC50) toxicity records for 291 species, including 24 endangered species (20 fish, four mussels). They showed that SSD approach is more protective, especially for endangered species, than applying safety factors.

With data collected for 16 insecticides, Maltby et al. [31] showed that the lower HC5 (95% protection with 95% confidence) estimate, based on SSDs with acute data, was protective of adverse ecological effects in freshwater mesocosms, but that the corresponding median HC5 (95% protection level with 50% confidence) was generally protective of single applications of insecticide but not of continuous or multiple applications. In the latter cases, a safety factor of

at least five should be applied to the median HC5. Comparison between SSDs based on acute data and semi-field studies showed that HC5 should be divided by a factor of three to protect ecosystems under long-term exposure to fungicides [32]. For herbicides, the lower limit of acute HC5 and the median value of chronic HC5 have been found to be protective of adverse effects in aquatic micro/mesocosms even under a long-term exposure regime [33]. In the present study, we showed through our simulations that there is a same probability for a chronic individual-based HC5 to be lower than a population-based HC5 than the opposite, with only slight differences related to the species composition. In general, population growth rate is equally or less sensitive to toxicant exposure than any of the individual life-history trait [34], as we showed here, but, in our simulations the slope for population-based SSDs was, in general, lower than the one for individual-based SSDs. In the case of copper, the population-based HC5 was higher than the chronic HC5, accounting for some related toxicity responses between species at population level. This suggests that population-based HC5 is theoretically as sensitive as chronic HC5 but could account for correlations in the toxicological responses of the different species, resulting in an HC5 value between chronic and acute HC5 values that may avoid overprotection. **We thus advise the derivation of population-based HC5, as soon as it is possible to derive such value with a relevant panel of species.**

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Figure legends

Fig. 1. Population growth rate and decrease of population growth rate as a function of exposure concentration for the 5 species of the copper case study.

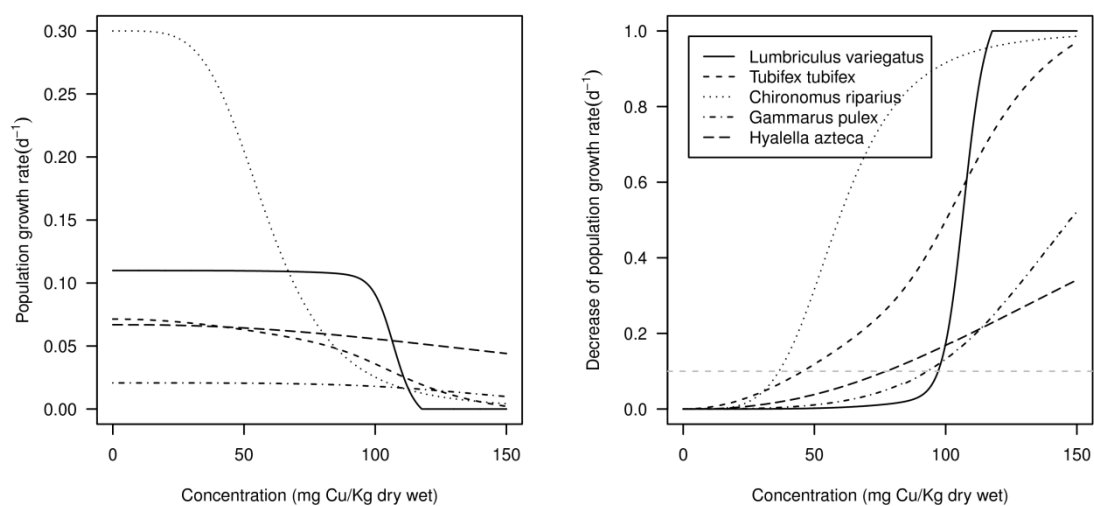


Fig. 2. SSD for the copper case study based on EC10 values at population level (Black points and full line) or at the individual level (white points and dotted line). Grey lines represent the value of the 5th percentile of the lognormal distribution.

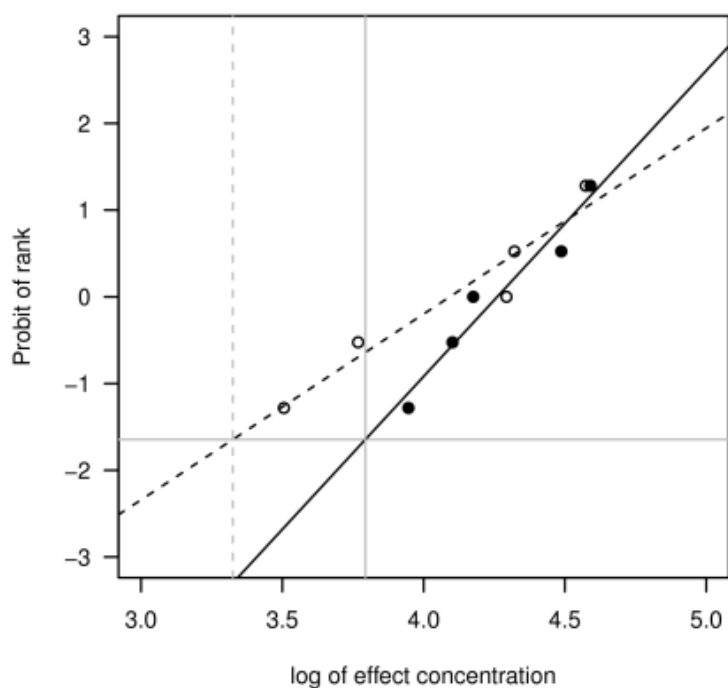


Table 1. Life cycle parameters used for the 5 species proposed by Ducrot et al. (2005)
 Juvenile survival (S_j), adult survival (S_a), age at first reproduction (t_j), time between reproduction events (t_a) and fecundity (F) and population growth rate (r).

Species	S_j	S_a	t_j (d)	t_a (d)	F	r	Reference
<i>Lumbriculus variegatus</i>	1	1	10	10	2	0.110	[8-9]
<i>Branchiura sowerbyi</i>	0.8	1	60	160	20	0.046	[7].
<i>Chironomus riparius</i>	0.9	0	15	0	100	0.299	[6]
<i>Valvata piscinalis</i>	1	0.99	56	41	120	0.086	[10]
		4					
<i>Cheumatopsyche</i>	0.09	0	172	0	70	0.011	[12]
<i>brevilineata</i>							

Table 2. EC10 values obtained for the 5 species of the copper case study (lowest EC10 ind is the lowest EC10 value obtained for the individual endpoints). All values are expressed in mg Cu/Kg dry wet. The slope values (*n*) for the dose responses with the Hill models are also indicated.

	<i>L. variegatus</i>	<i>T. tubifex</i>	<i>C. riparius</i>	<i>G. pulex</i>	<i>H. azteca</i>
EC10 pop	98.5	51.7	60.4	65.0	88.85
Lowest EC10 ind	96.9	43.3	33.3	73.2	75.3
EC10 growth		43.3	92.5	102	75.3
EC10 reproduction	96.9	79.2			
EC10 juvenile survival	126	160	33.3	73.2	135
EC10 adult survival	126	160			
<i>n</i> EC10 growth		2.1	4.5	5.9	2.3
<i>n</i> EC10 reproduction	27.4	10.3			
<i>n</i> EC10 juvenile survival	4.3	3.1	3.8	3.0	2.6
<i>n</i> EC10 adult survival	4.3	3.1			

Table 3. Life cycle parameters used for the 5 species in the copper case studies. (Juvenile survival (S_j), adult survival (S_a), age at first reproduction (t_j), time between reproduction events (t_a) and fecundity (F)) and population growth rate (r)

Species	S_j	S_a	$t_j (d)$	$t_a (d)$	F	r	Reference
<i>Lumbriculus variegatus</i>	1	1	10	10	2	0.110	[8-9]
<i>Tubifex tubifex</i>	1	1	40	28	15	0.071	[17]
<i>Chironomus riparius</i>	0.9	0	15	0	100	0.299	[6]
<i>Gammarus pulex</i>	0.7	1	130	13	5	0.021	[18-19]
<i>Hyalella azteca</i>	0.9	1	30	28	7	0.067	[20]